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Nobel Celebrates the Neurosciences: Modulatory Signaling in the Brain

Essay

One of the ultimate frontiers for mankind is the elucidation of the function of the mind. It seems therefore appropriate that in this millennial year—and at the end of the "decade of the brain"—the Nobel prize in Physiology or Medicine has recognized the key discoveries of three scientists who have played a significant role in pioneering this frontier. Arvid Carlsson, Paul Greengard, and Eric Kandel have followed very different scientific paths, but in complementary and synergistic ways have made seminal contributions to the current understanding of information processing within the brain.

Modern neuroscience was born at the end of the nineteenth century, when the development of new histological methods that could reveal the complexity of neuronal architecture triggered a systematic analysis of the structure of the nervous system and of the interconnectivity of nerve cells. For their seminal work in this area, two neuroscientists, Camillo Golgi, who discovered the "black reaction" while tinkering with cytochemical techniques in the lab of a small psychiatric hospital, and Santiago Ramon y Cajal, who applied this method in a monumental work of descriptive neuroanatomy, shared one of the first Nobel prizes (in Physiology or Medicine) in 1906. During the following decades a major impetus of neuroscience focused on the refinement of this structural work and on the elucidation of the ways in which electrical signals (impulses) are propagated along nerve fibers and transmitted from cell to cell. A vision of the nervous system emerged as a network of electrical cables interconnected to each other at synapses. Neurons were the building blocks of this network, and synaptic transmission, thought of as the mechanism for fast point-to-point communication, was considered to be the primary form of signaling among nerve cells. This era culminated with two other fundamental achievements also celebrated by several Nobel prizes: the description of the ionic basis of neuronal excitability by Hodgkin and Huxley and the elucidation of the mechanisms of chemical transmission at central and peripheral synapses, in particular by the work of Eccles and Katz. Collectively, this research revealed the electrical properties of nervous tissue and defined the road map followed by electrical signals. The hardware of the neuronal computer had thus been elucidated. Then came the task of understanding how chemical and electrical signals are interconverted, and determining how the function of this hardware is regulated to achieve the remarkable plasticity of neuronal function. Carlsson, Greengard, and Kandel have been key figures in the pursuit of this new frontier.

Arvid Carlsson, a Swedish neuroscientist and the founding father of the distinguished Swedish School of Neuropsychopharmacologists, discovered an important new neurotransmitter, dopamine, in the brain and showed its role in motor control, behavior, and higher brain function. Furthermore, he demonstrated the involvement of dopaminergic neuronal signaling in neurological and psychiatric diseases and in their treatment. In the mid 1950s, Carlsson, then at the University of Lund, Sweden, had become interested in the biology of brain amines during a five-month period spent in the laboratory of Bernard Brodie at the National Institutes of Health in the USA. After his return to Lund he developed the first useful methodology for measuring brain dopamine and its metabolites with high sensitivity. Using this technique he found that the distribution of dopamine, a known precursor of the neurotransmitter noradrenaline, was inconsistent with it simply being a precursor of noradrenaline and postulated its independent role as a chemical messenger in brain. Work from his laboratory also established the critical role of dopamine in the basal ganglia of the brain, a region crucially important in the control of body musculature.

Important experimental support for his hypothesis that dopamine serves as a chemical messenger in the nervous system came from his work with reserpine, a natural alkaloid derived from the plant *Rauwolfia serpentina*. Reserpine, which had been used in ancient Hindu medicine to treat a variety of conditions, had been introduced in the western world in the 1950s for its sedative and antihypertensive effects. Carlsson had contributed to the demonstration that reserpine induces depletion of intracellular stores of amines, a property now known to be explained by its property of binding and inhibiting the monoamine transporter of secretory vesicles. He then went on to show that administering reserpine to rodents induced movement dysfunction similar to that characteristic of Parkinson's disease. He further demonstrated that the motor impairment was due to the specific depletion of dopamine in brain, and showed that L-DOPA, a precursor of dopamine that can cross the blood–brain barrier, could relieve some of the motor impairment in parallel with restoration of dopamine levels (but not norepinephrine levels) in brain. This observation led to the discovery by the Austrian neurologist Hornykiewicz that dopamine levels are reduced in the basal ganglia of patients with Parkinson's disease and prompted the administration of L-DOPA to these patients, with a resulting striking regression of the symptoms. Although these dramatic effects tend to decrease with time, the development of L-DOPA therapy for Parkinson's disease represented a major triumph of neuropharmacology, and it is still a widely used treatment for this debilitating neurological disorder.

In 1959, Carlsson moved to the University of Göteborg in Sweden where he served as a Professor of Clinical Pharmacology. There he followed up on the clinical observation of Nathan Kline and colleagues that reserpine had antipsychotic properties (extracts of *Rauwolfia* roots had been used in India to treat insanity), and explored a more general link between antipsychotic drug action and dopamine function in brain. He found that, in contrast to reserpine, two other antipsychotic drugs, chlorpromazine and haloperidol, that belong to two distinct chemical classes, did not deplete brain dopamine but increased its turnover; yet they caused similar behavioral changes as reserpine. He speculated that the shared property of these drugs, underlying their antipsychotic effect, was a disruption of dopaminergic signaling. With far-reaching insight, he suggested that chlor-

promazine and haloperidol act by blocking a "dopamine receptor" and speculated that such block could induce, via a feedback mechanism, a compensatory activity of dopamine secreting neurons, thus explaining the increase in dopamine turnover. His predictions were subsequently confirmed by pharmacological and electrophysiological studies from other groups and by the discovery of a feedback regulatory neuronal circuit that controls the activity of midbrain dopaminergic neurons projecting to the caudate–putamen regions of the basal ganglia.

Finally, Carlsson and colleagues made another important contribution to the treatment of mental illness with the demonstration that one class of antidepressants acts by blocking the uptake into the cell of another brain monoamine, serotonin, and the subsequent development of zimelidine, the first selective serotonin reuptake inhibitor. Beyond his specific discoveries, Carlsson's studies helped to launch a new era of neuropsychopharmacology based on the rational development of drugs that affect interneuronal signaling. An interesting feature of Carlsson's papers is that they are strikingly short, at least when compared to present standards. They remind us that relatively few words are needed to explain truly fundamental findings. It is also interesting to note that some of Carlsson's conclusions were highly indirect (due to the technical limitations of his day), yet impressively on target. His achievements demonstrate the value of a scientific path that emphasizes creative insight in addition to rigorous experimentation and exploration.

Carlsson's findings prompted a search for the mechanisms that transduce the signal of dopamine in target cells, and turned out to be very influential for subsequent studies by Paul Greengard. Greengard entered graduate school at Johns Hopkins in 1949 to pursue a degree in biophysics. Early in his studies, a seminar by Hodgkin had a major impact on his plans. He left the talk convinced that the fundamental questions concerning the ionic basis of neuronal excitability, which could be addressed with the methodology available at the time, had been solved. He predicted that it would take many years before a new level of understanding of these phenomena could be achieved by the use of biophysical methods. Thus, he decided to search for novel approaches to study the nervous system and developed an interest in biochemistry. A "functional" biochemistry of the brain was practically nonexistent at that time and was very much a matter of establishing a new discipline.

After 5 years of postdoctoral training in England, Greengard became Director of Biochemistry at Geigy where he studied several aspects of nervous tissue metabolism and pharmacology. Eventually, he focused on the mechanisms of action of hormones, which culminated in a sabbatical at Vanderbilt University. There he became familiar with the work of Earl Sutherland, who had discovered the role of cAMP as an intracellular mediator of the action of glucagone and adrenaline. Soon afterwards, Edward Krebs isolated cAMP-dependent protein kinase, which phosphorylates and activates phosphorylase kinase, thereby mediating the actions of cAMP on glucose metabolism. Greengard saw the potential general implications of these findings and formulated two hypotheses that eventually represented the cornerstone of his subsequent scientific career. The first was that reversible protein phosphorylation is a general mechanism through which intracellular second messengers achieve their effects. The second was that brain neurotransmitters, like hormones, could act by regulating intracellular second messengers and second messenger–dependent protein phosphorylation cascades.

Upon returning to academia as a Professor of Pharmacology at Yale in 1968, Greengard set out to test these ideas. Both hypotheses turned out to be correct beyond the most optimistic expectations. After discovering cAMP protein kinase in the brain and showing that it was enriched in synaptic fractions, he searched for other second messenger-regulated protein kinases. He showed that not only cAMP, but also cGMP, stimulates a specific protein kinase and that many actions of Ca^{2+} are mediated by Ca^{2+}-calmodulin-dependent protein kinases. These findings have been equally important to the general field of intracellular regulation as they have to the field of neuroscience. He also searched for an effect of brain neurotransmitters on intracellular second messengers and found several examples of such effects. Here is where his path crossed for the first time that of Arvid Carlsson. He discovered the potent stimulatory effect of dopamine on adenylyl cyclase in the brain, and implicated the dopamine signaling pathway in the actions of antipsychotic drugs.

But Greengard's ultimate goal was to determine whether protein phosphorylation could affect critical parameters of neuronal function. Both through work carried out in his laboratory and through powerful collaborations (including a collaboration with Eric Kandel), he repeatedly found striking validations of the hypothesis that protein phosphorylation can regulate neurotransmitter release and ion channel permeability, the key mediators of fast neuronal signaling. Furthermore, he carried out a systematic search of protein kinase substrates for second messenger–dependent kinases in the nervous system. The molecular and functional characterization of these proteins and of the signaling cascades controlling their function ultimately became his life-long commitment, first at Yale and then at the Rockefeller University. At first, it was just a few weak bands of proteins in SDS-polyacrylamide gels, then the field blossomed into a multitude of substrate proteins that participate directly or indirectly in nearly all key neuronal processes. Through these studies Greengard and his colleagues contributed to many areas of neurobiology, ranging from the cell biology of synapses to molecular mechanisms of neurological and psychiatric diseases.

Once again, the dopaminergic pathway turned out to be a very powerful model system for two reasons. First, the relatively simple histological structure of the portion of the basal ganglia which receive the primary dopaminergic input of the brain (the caudate and the putamen) allowed a systematic analysis of downstream targets of dopamine receptors without the complication resulting from cell heterogeneity. In this region the dopaminoceptive "medium spiny neuron," which is innervated by dopaminergic axons originating from the substantia nigra in the midbrain, represents by far the predominant neuronal cell type. Second, it turned out that most (and perhaps all) actions of dopamine receptors are mediated by intracellular second messengers or G protein–

coupled signaling, and not directly by ion channels. These studies led to the discovery of a cytosolic network of signaling proteins (protein kinases, protein phosphatases, the phosphatase inhibitor DARPP-32) that functions as an integrator of all the signals converging onto dopaminoceptive cells and eventually controls the response to synaptic stimuli of all the main cellular effector systems, such as ion channels, the secretory apparatus, the cytoskeleton, and the transcription and translating machineries. DARPP-32, in particular, has emerged as a central regulator of the biology of these cells. The detailed characterization of its multi-site phosphorylation, of its interplay with protein kinase and protein phosphatase cascades, as well as of its upstream regulators and downstream effectors, represent an impressive body of work which gives us a glimpse of how cytosolic proteins can participate in signal integration. Furthermore, given the critical role of dopaminergic transmission in a variety of human diseases, such as schizophrenia, Parkinson's disease, and drug addiction, this information offers new potential targets for therapeutic interventions. More than other areas of research touched by Greengard's work, his investigation of the intracellular transduction of the dopamine signal encapsulates his fundamental contribution to neuroscience: the discovery that the cell cytoplasm does not play only an ancillary role to the signaling properties of the neuronal membrane, but has a key function of its own in information processing.

Today's student may find it hard to believe that Greengard's original hypotheses were highly innovative and controversial at the time they were formulated. While the existence of hundreds of protein kinases is now well established, only a handful of them had been discovered at the time and they were thought to play a specific role in glycogen metabolism in liver and muscle. The scientific community had not yet appreciated the remarkable evolutionary conservation of cell physiology from unicellular organisms to neurons. The idea that biochemical mechanisms used by cells to regulate metabolism could also be important for signal processing and signal integration in the brain initially encountered strong resistance. In a culture dominated by the idea that neuronal communication was based entirely on electrical signals generated by ion fluxes, the time needed for biochemical cascades seemed to be incompatible with the speed of neuronal signaling. As it turned out, Greengard and his colleagues were discovering a new layer of signaling that works in parallel and synergistically with the process of electrical signaling and is equally important. This additional layer of signaling, which is mediated by intracellular messages, operates over longer time scales and has both short and long lasting actions on virtually all neuronal functions, including fast signaling mechanisms. The concepts established by Greengard with his early work in this area laid the foundation for the proper understanding of subsequent discoveries in neurobiology, such as the cosecretion of neurotransmitters which mediate fast and slow responses at synapses, and the cooperation of ion channel receptors and G protein–coupled receptors in the transduction of signals mediated by classical neurotransmitters. Extending the metaphor that compares the brain to a computer, one can say that Paul Greengard helped to pioneer the study of the "software" of the nervous system.

Three years after Greengard entered Johns Hopkins to begin his graduate work, Eric Kandel graduated from Harvard College and began his training in medicine at New York University. At the outset of his scientific career, Eric Kandel was a man with a mission: to find an experimental system that would allow him to ask fundamental questions about learning and memory at the level of circuits, cells, and synapses. After graduating from New York University in 1956, he went to the NIMH to begin his search. There he met Alden Spencer, who became a life-long friend and colleague. Spencer shared Kandel's passion for understanding learning and memory, and together they embarked on a series of classic studies examining the synaptic organization of the hippocampus, a brain structure that was clearly implicated in learning and memory. While this collaboration proved extremely fruitful for the analysis of the properties of hippocampal neurons, it soon became apparent to both Kandel and Spencer that the hippocampus was, at the time, just too complicated a system for a detailed analysis of learning. Thus each went his separate way: Spencer turned to an investigation of the spinal cord, and Kandel turned to the marine mollusc *Aplysia*.

Kandel had learned of *Aplysia* from a visit of Ladislav Tauc to the NIMH. A few years later, after finishing a psychiatric residency at Harvard Medical School, Kandel went to Tauc's laboratory in Paris to continue his quest for the right experimental system. And he found it. While working with Tauc, Kandel developed a creative idea: he reasoned that Pavlov and Thorndike, who at the turn of the twentieth century had carried out landmark studies of learning and memory, were essentially giving instructions to animals. Why not do the same to the simple nervous system of *Aplysia*? So he did just that. Using the input from nerves as conditioning and reinforcement pathways, Kandel and Tauc carried out a seminal series of experiments that put *Aplysia* on the map as a preparation that could be used for the synaptic analysis of learning.

After 18 months in Paris, Kandel returned to Harvard for a year and then to New York University School of Medicine. There he launched an enterprise that completely changed the field of learning and memory. He built on the synthesis of three main ideas: first, as alluded to above, he appreciated the lesson from Pavlov that learning can be studied in a rigorous way using a reflex system; second, he reasoned that a simple reflex would be the type of behavior best suited for the analysis of learning and memory at a mechanistic level; and third, he recognized that a simple animal such as *Aplysia*, which had a highly accessible nervous system, could possess just the reflex system he was looking for. Thus, along with his colleagues, Kandel turned to a detailed analysis of a simple protective reflex, the gill withdrawal reflex, in *Aplysia.* He delineated many of the critical circuit elements in the reflex pathway and showed that this simple reflex exhibited three different forms of nonassociative learning: habituation, dishabituation, and sensitization. Most importantly, focusing his attention on the connections between sensory neurons and motor neurons involved in this reflex pathway, Kandel directly showed, for the first time, that learning and memory

involved changes in synaptic strength. For example, the general arousal and increased behavioral responsiveness of a sensitized animal were accompanied by an increase in the release of transmitter from the sensory neurons. This series of studies caused a sea change in the field, for they pointed the way to establishing direct links between specific forms of synaptic plasticity and the acquisition and storage of memories in identifiable neural circuits.

Building on this discovery, Kandel set out to identify the cellular and molecular substrates of learning and memory in *Aplysia,* first at New York University, and subsequently at Columbia University College of Physicians and Surgeons, where in 1984 he became a University Professor and Senior Investigator at the Howard Hughes Medical Institute. One of his early achievements toward this goal centered on the elucidation of the mechanisms underlying the changes in synaptic strength that contribute to short-term memory for sensitization. In this area, Kandel's pursuits intersected importantly with the work of Paul Greengard. Kandel had shown that sensitization is mediated in part by the amine serotonin. He went on to show that serotonin increases levels of cAMP in the presynaptic cell and induces phosphorylation of preexisting proteins via the cAMP-dependent protein kinase, PKA. In collaboration with Greengard he showed that direct injection of the catalytic subunit of PKA into the sensory neurons induced an increase in transmitter release similar to the increase observed during sensitization, and that a main role in these effects was played by the PKA-mediated closure of K^+ channels. These studies played a seminal role in the field of channel regulation. In addition they provided the fundamental demonstration that covalent modification of proteins could provide a general biochemical mechanism for short-term memory.

But what about long-term memory? While it had long been appreciated that short-term and long-term memories differed behaviorally, it was the work of Kandel and colleagues that brought this important problem into register with modern molecular biology. He showed that long-term memory for sensitization in *Aplysia* involved a transcription-dependent step mediated, at least in part, by the translocation of PKA to the nucleus, regulation of gene transcription and the synthesis of new proteins. Some of these proteins are then targeted to the synapse where they participate in the long-lasting modification of synaptic strength. These modifications ranged from changes in adhesion molecules on the surface of the sensory neuron to the growth of new synaptic connections. Transcription factors regulated by cAMP-dependent phosphorylation, such as the transcriptional activator CREB-1a, and the transcriptional repressor CREB-2, play a central role in these processes. The broad impact of this work is demonstrated by more recent genetic and molecular evidence from other laboratories demonstrating the critical role of both activator and repressor isoforms of CREB in long-term memory in *Drosophila* and mice. Thus, the studies by Kandel have provided deep and lasting insights into the molecular architecture of enduring memories.

Another milestone in Kandel's career was the demonstration that *Aplysia* could exhibit a form of associative learning, Pavlovian conditioning. He showed that the mechanism contributing to Pavlovian conditioning in the gill withdrawal reflex involved, at least in part, an activity-dependent amplification of some of the cellular processes in the sensory neurons that underlie sensitization. These studies helped to elucidate some of the mechanistic similarities between associative and nonassociative learning.

Finally, during the past decade Kandel has also returned to his roots as a young scientist by taking a fresh look at synaptic plasticity in the mammalian hippocampus. Using the cellular and molecular insights gained from *Aplysia*, he has studied a form of synaptic plasticity, long-term potentiation (LTP), in this system. Several laboratories had previously provided seminal insights into the mechanisms of induction, expression, and maintenance of a form of LTP that lasts on the order of 3 hr. Kandel turned his attention to another form of LTP that lasts several hours longer, and showed that it requires both transcription and translation, and is PKA dependent. Moreover, he showed that mice expressing a dominant-negative transgene that inhibits PKA show defective long-lasting LTP and defective long-term memory. This single example serves to illustrate the power of combining molecular biology with cellular physiology and behavior in the analysis of learning and memory.

Kandel's career, like those of the other two Nobel laureates, is characterized by many great experimental contributions to the field of neuroscience. But eclipsing any of his single achievements was his powerful demonstration that the fundamental properties of learning and memory are experimentally tractable problems that can be approached with the tools of cellular and molecular biology. From his work we have learned that complex functions of the brain are not the result of special properties unique to the nervous system, but are the products of the very same molecular and biochemical mechanisms that all cells use in the regulation of their responses to their environment. This in turn reflects another fascinating level of conservation from unicellular organisms to neurons. The analysis of the cellular and molecular architecture of any system can uncover some of the basic tools utilized by evolution to promote adaptive change throughout the animal kingdom.

The decision of the Nobel Committee recognizes the common thread that links the work of Carlsson, Greengard, and Kandel. Carlsson's studies provided a first demonstration that the effect of amines in the brain goes far beyond an effect on metabolism and vasculature and provided an impetus toward an elucidation of their mechanism of action. Greengard's implication of dopamine in the regulation of neural function via intracellular second messengers was, in turn, an important foundation both for his own subsequent research as well as for some of the early studies of Kandel. There is a beautiful convergence and synergy between the work of Greengard and Kandel. Greengard's study of brain biochemistry has laid the ground for the understanding of the molecular basis of complex neuronal functions. Coming from another direction, from behavior to molecules, Kandel has shown that changes in behavior ultimately reflect changes in protein function and gene expression. The unifying motif of all these contributions is that there is much more to brain than ion fluxes and electrical signaling. Thus, a century after the first systematic de-

scription of the neuronal structure of the brain, we have begun to appreciate the order that governs the intracellular world of the neuron and its critical role in the regulation of neuronal function. New avenues toward both the explanation and the therapy of neurological and psychiatric diseases have been opened.

A final comment on this year's Nobel laureates is warranted. The collective scientific achievements of the three awardees are extraordinary. But perhaps of equal importance to the field of neuroscience is their contribution of a genuine excitement not just for the acquisition of facts, but for the process of science. Each in his own way has inspired subsequent generations of young scientists to think beyond the obvious, to stretch contemporary ideas into the future, to hold one's scientific ground when one's ideas don't fit conventional wisdom, and to take risks. These are Nobel lessons indeed.

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